Amer. J. Bot. 66:1138–1150. 1976; Whittier, Canad. J. Bot. 55:563–567. 1977). The gametangia of *Lycopodiella appressa* (F.Lloyd & L.Under.) Cranfill and *Lycopodiella cernua* (L.) Pichi-Serm. have essentially the same sizes as those of *L. prostrata*. The gametangia of *L. prostrata* are typical for *Lycopodiella*.

The development of the other types of gametophytes of the Lycopodiaceae is quite different from that found in *Lycopodiella*. The mature gametophyte of *Phylloglossum* is photosynthetic but it starts out as a subterranean, mycorrhizal gametophyte that is negatively gravitropic. After its exposure to light at the soil surface it becomes a green, bilaterally symmetrical, tuberous gametophyte lacking photosynthetic lobes (Whittier & Braggins, Amer. J. Bot. 87:920–924. 2000).

The remaining gametophytes of the Lycopodiaceae are subterranean, mycorrhizal, and nonphotosynthetic. Their development is initiated underground by the dark germination of their spores and requires a mycorrhizal association for continued growth. Early growth forms a solid, teardrop-shaped gametophyte that gives rise to the four other gametophyte shapes found in the Lycopodiaceae. Larger teardrop-shaped gametophytes develop ring meristems that form the radially symmetrical disk- and carrot-shaped gametophytes of *Lycopodium* (Whittier, Canad. J. Bot. 55:563–567. 1977; Whittier, Bot. Gaz. 142:519–524. 1981).

The uniaxial, dorsiventral, strap-shaped gametophyte of the terrestrial *Huperzia* species lacks a ring meristem. The meristem arises from a portion of the apical region of a larger teardrop-shaped gametophyte (Bruchmann, Flora 101:220–267. 1910). This meristem occurs in a subterminal groove overarched by young dorsal tissue on these strap-shaped gametophytes. With the epiphytic *Huperzia* species, the teardrop-shaped gametophyte enlarges and grows into the branched, cylindrical, mycorrhizal gametophyte (Whittier unpublished).

The gametophyte of *L. prostrata* has the typical structure and development of *Lycopodiella* gametophytes; thus it is different from the other gametophyte types of the Lycopodiaceae.—Dean P. Whittier, Department of Biological Sciences, Box 1634, Vanderbilt University, Nashville, TN 37235-1634, and Richard Carter, Department of Biology, Valdosta State University, Valdosta, GA 31698-0015.

Three New Flavonoid Glycosides, Kaempferol 3-*O*-(caffeoylrhamnoside), Apigenin 4′-*O*-(caffeoylglucoside) and 4′-*O*-(feruloylglucoside) from *Dryopteris villarii*.—Ten flavonol *O*-glycosides (based on kaempferol and quercetin), two flavanone *O*-glycosides (based on naringenin and eriodictyol) and three *C*-glycosylflavones (vitexin, vitexin 7-*O*-glucoside and orientin) have previously been identified by Hiraoka (Biochem. Syst. Ecol. 6: 171-175. 1978) in eighteen *Dryopteris* species whereas 3-desoxyanthocyanins have been found in red sori of *Dryopteris erythrosora* (Eat.) Kuntze by Harborne (Phytochemistry 5: 589–600. 1966). In addition kaempferol 7-*O*-(6″-succinyl-

glucoside) was found in four *Dryopteris* species and an unusual flavan was isolated from *Dryopteris filix-mas* (L.) Schott as shown in a review by Markham (pp. 427–468, *in* J.B. Harborne ed., *The Flavonoids, Advances in Research since 1980*. Chapman and Hall, London and New York. 1988). Eighteen flavonoids (14 flavonol glycosides, one flavone glycoside and three aglycones) have been found recently in *Dryopteris villarii* by Imperato (Amer. Fern J. 96: 93–96. 2006; Amer. Fern J. 97(2): 124–126. 2007; Nat. Prod. Commun. 2: 909–912. 2007).

This paper deals with identification of three flavonoids (I–III) from aerial parts of *Dryopteris villarii* (Bellardi) Schinz & Thell collected in the Botanic Garden of the University of Naples (Italy). The fern was identified by Dr. R. Nazzaro (Università "Federico II", Naples); a voucher specimen (*NAPEA 3496*) has been deposited in Herbarium of Dipartimento di Biologia, Università "Federico II", Naples, Italy (NAP).

Flavonoids (I–III) were isolated from an ethanolic extract of aerial parts of *Dryopteris villarii* by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% AcOH (acetic acid) and BEW (*n*-butanol-ethanol-water, 4:1:2.2). Further purification was carried out by Sephadex LH-20 column chromatography eluting with methanol.

Color reactions (brown to yellow in UV+NH_3), R_f values on Whatman N.1 paper (0.75 in BAW; 0.23 in 15% AcOH; 0.08 in water) and UV spectral analysis in the presence of usual flavonoid shift reagents (λ_{max} (nm) (MeOH) 266, 324; +$AlCl_3$ 274, 303, 347, 398; +$AlCl_3$/HCl 274, 300, 343, 396; +NaOAc 272, 300, 370; +NaOMe 272, 325, 395) suggested that flavonoid (I) may be a flavonoid glycoside with free hydroxyl groups at positions 5, 7 and 4′ (shifts with $AlCl_3$/HCl, NaOAc and NaOMe respectively); in addition flavonoid (I) may be acylated with a hydroxycinnamic acid since the UV spectrum of hydroxycinnamic acid is superimposed on the flavonoid spectrum as shown in a review by Harborne and Williams (pp. 376–441 *in* J.B. Harborne, T.J. Mabry, H.Mabry, eds. *The Flavonoids*, Chapman and Hall, London. 1975). Both total acid hydrolysis (2 N HCl; 2 hr at 100°C) and controlled acid hydrolysis (10% AcOH, 3.5 hr under reflux) gave kaempferol and L-rhamnose whereas alkaline hydrolysis (2N NaOH, 2 hr at room temperature in a sealed tube) gave 3, 4-dihydroxycinnamic acid (caffeic acid) and kaempferol 3-*O*-rhamnoside. CID (collision induced dissociation) mass spectrum (negative mode) gave a quasi-molecular ion $[M-H]^-$ at m/z 593 and fragment ions at m/z 431 (kaempferol 3-*O*-rhamnoside) and m/z 285 (kaempferol). These results show that flavonoid (I) is kaempferol 3-*O*-(caffeoylrhamnoside), a new natural product (Fig. 1).

Color reactions (brown to yellow in UV+NH_3), R_f values on Whatman N.1 paper (0.78 in BAW, 0.21 in 15% AcOH, 0.04 in water) and UV spectral analysis in the presence of usual shift reagents (λ_{max} (nm) (MeOH) 262, 322; +$AlCl_3$ 272, 305 (sh), 340, 383; +$AlCl_3$/HCl 270, 294 (sh), 326, 386 (sh); +NaOAc 268, 340; +NaOMe 271, 347) showed that flavonoid (II) may be a flavonoid glycoside with free hydroxyl groups at positions 5 and 7 (shifts with $AlCl_3$/HCl and NaOAc respectively). In addition flavonoid (II) may be acylated with a hydroxycinnamic acid since the UV spectrum of hydro-

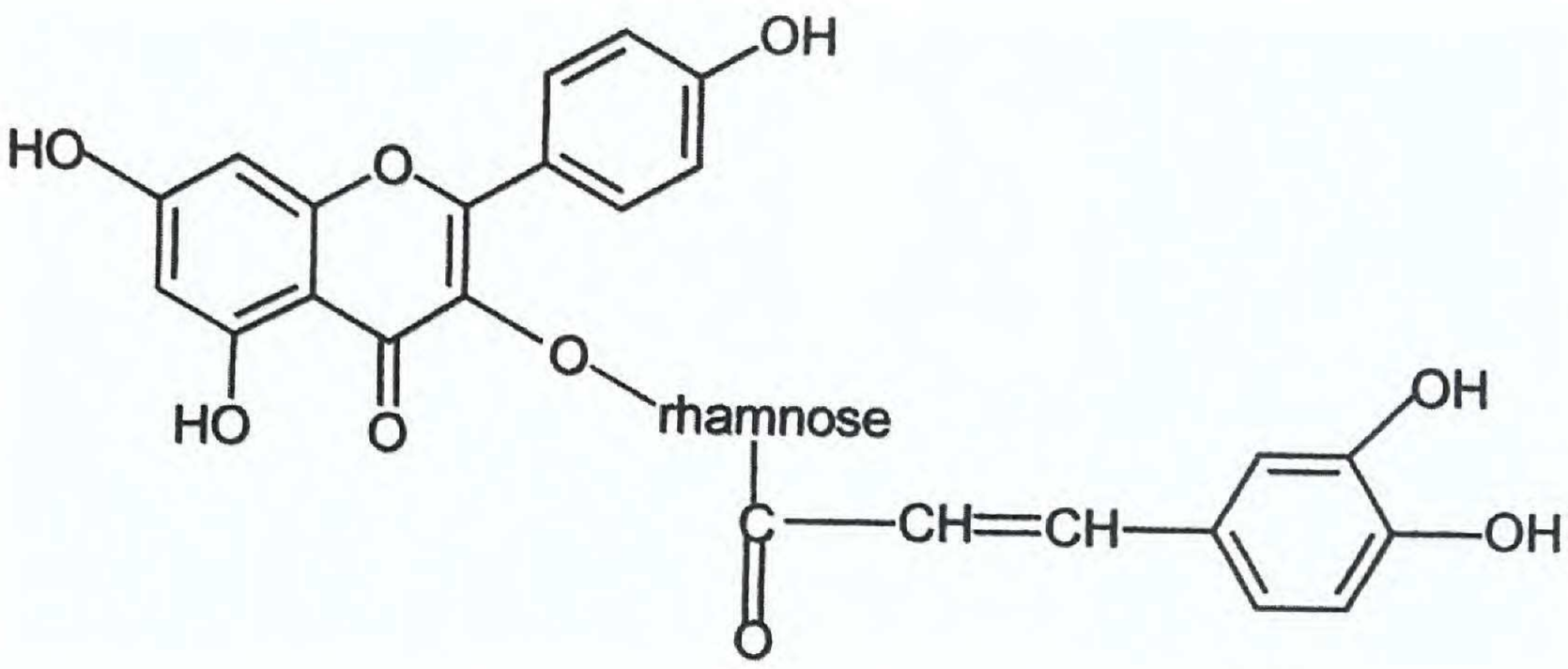

FIG. 1. Kaempferol 3-*O*-(caffeoylrhamnoside).

xycinnamic acid is superimposed on the flavonoid spectrum (Harborne and Williams, 1975). Both total acid hydrolysis (2N HCl; 1 hr at 100°C) and controlled acid hydrolysis (10% AcOH; 3.5 hr under reflux) gave apigenin and D-glucose whereas alkaline hydrolysis (2 N NaOH; 2 hr at room temperature in a sealed tube) gave apigenin 4′-*O*-glucoside and 3, 4-dihydroxycinnamic acid (caffeic acid). CID (collision induced dissociation) mass spectrum (negative mode) showed a quasimolecular ion at m/z 593 $[M-H]^-$ and fragment ions at m/z 431 (apigenin 4′-*O*-glucoside), m/z269 (apigenin) and m/z 253 (glucosylated B-ring). These results show that flavonoid (II) is apigenin 4′-*O*-(caffeoylglucoside), a new natural product (Fig. 2).

Color reactions (brown to yellow in UV+NH_3), R_f values on Whatman n.1 paper (0.72 in BAW; 0.26 in 15% HOAc; 0.04 in water) and UV spectral analysis with the usual shift reagents (λ_{max} (nm) (MeOH) 263, 319; +$AlCl_3$ 272, 305, 340, 382; +$AlCl_3$/HCl 270, 294, 326, 380 (sh); +NaOAc 268, 340; +NaOMe 271, 351) suggested that flavonoid (III) may be a flavonoid glycoside with free hydroxyl groups at positions 5 and 7 (shifts with $AlCl_3$/HCl and NaOAc respectively); in addition flavonoid (III) may be acylated with hydroxycinnamic acid since the UV spectrum is superimposed on the flavonoid spectrum (Harborne and Williams, 1975). Both total acid hydrolysis (2N HCl; 1 hr at 100°C) and controlled acid hydrolysis (10% AcOH; 3.5 hr under reflux) gave apigenin and D-glucose whereas alkaline hydrolysis (2N NaOH; 2 hr at room temperature in a sealed tube) gave 3-methoxy-4-hydroxy-cinnamic acid (ferulic acid) and apigenin 4′-*O*-glucoside. The electrospray mass spectrum exhibits a pseudomolecular ion at m/z 632 $[(M+H)+Na]^+$ and fragment ions at m/z 455 (apigenin glucoside+Na) and 271 (apigenin). These results show that flavonoid (III) is apigenin 4′-*O*-(feruloylglucoside), a new natural product (Fig 2).

As shown in a review by Williams (pp. 749–856 *in* O.M. Andersen and K.R. Markham eds., *Flavonoids*, *Chemistry*, *Biochemistry and Applications*, CRC Press, London, New York. 2006) flavonoids having an acyl group linked to a carbohydrate attached at position 4′ of B-ring (as flavonoids (II) and (III)) are rare natural products. Such compounds have previously been reported by

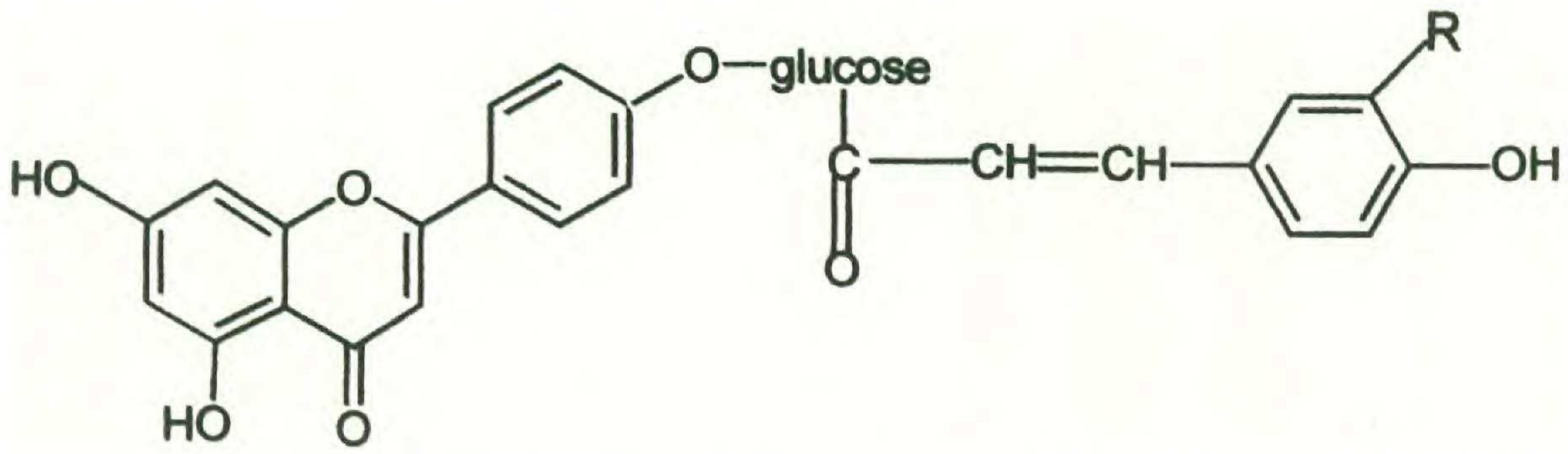

FIG. 2. Apigenin 4′-*O*-(caffeoylrhamnoside). R=OH; Apigenin 4′-*O*-(feruloylglucoside). R=OCH_3.

Horie *et al.* (Phytochemistry 25: 2621–2624. 1986) who isolated 6, 8-dihydroxyluteolin 6, 8, 3′-trimethyl ether 4′-*O*-(6″-(3-hydroxy-3-methylglutaryl) glucoside) (sudachiin B) from *Citrus sudachi* green peel (Rutaceae) and by Stockmalia *et al.* (Phytochemistry 57: 1223–1226. 2001) who found apigenin 4′-*O*-(2″ feruloylglucuronosyl (1→2) glucuronide) from aerial parts of *Medicago sativa* L. var. *artal* (Leguminosae). The first occurrence of these compounds in ferns has been described by Imperato (Nat. Prod. Commun. 2: 909–912. 2007) who identified apigenin 4′-*O*-(*p*-coumaroylglucoside) in aerial parts of *Dryopteris villarii*. The presence of acylated flavonoid glycosides previously reported in *Dryopteris villarii* by Imperato (Amer. Fern J. 96, 93–96. 2006; Nat. Prod. Commun. 2: 909–912. 2007; Amer. Fern J. 97(2): 124–126. 2007) and identification of three further acylated flavonoid glycosides (I–III) in *Dryopteris villarii* show that this fern has a number of acylated flavonoid glycosides which are generally absent from *Dryopteris* species with the exception of kaempferol 7-*O*-(6″ succinylglucoside) in four *Dryopteris* species and a flavan acetate in *Dryopteris filix-mas* (L.) as shown in a review by Markham (1988).

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